EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 2
804-810
Year: 2018

IN SILICO ANALYSIS OF NATURAL ANTIVIRAL COMPOUNDS AGAINST HIV

Vemula Vani[1*], Keerthika V[2], Manisha Lahiri[3], Monisha Sarkar[4]

[1,2,3&4]Department of Microbiology, Ramaiah College of Arts, Science and Commerce, Bangalore- 560054.

***Corresponding Author: Dr. Vemula Vani**
Department of Microbiology, Ramaiah College of Arts, Science and Commerce, Bangalore- 560054.

Article Received on 10/12/2017 **Article Revised on 31/12/2017** **Article Accepted on 21/01/2018**

ABSTRACT
HIV is a virus that attacks the immune system which is our body's natural defense against illness. The virus destroys a type of white blood cells in the immune system called T-helper cells, and makes copies of itself inside these cells. The T-helper cells are also referred to as CD4 cells. As HIV destroys more CD4 cells and makes more copies of itself, it gradually breaks down a person's immune system. HIV-1 is the most widespread type worldwide. It is related to viruses found in chimpanzees and gorillas living in Western Africa. The viruses may further divided into groups. The HIV-1 group M viruses predominate and are responsible for the AIDS pandemic. Group M can be further subdivided into subtypes based on genetic sequence data. Some of the subtypes are known to be more virulent or are resistant to different medications. Currently, antiretroviral therapies (ART) available for symptomatic treatment of AIDS are quite expensive and are associated with rapid emergence of drug resistance. ART is the combination of several antiretroviral medicines used to slow the rate at which HIV multiplies in the body. The objectives of this study are to select natural antiviral compounds from the medicinal plants based on review of literature and to test the effect of these antiviral compounds targeting the proteins of HIV by *in-silico* methods. Thirty six natural antiviral compounds were selected based on review of literature. These compounds were screened based on Lipinski Rule of Five. Out of thirty six compounds, twenty nine were found to be positive. These twenty nine compounds were tested for their affinity against four potential drug targets (GP 41, GP 120, protease and reverse transcriptase) of HIV-1. Docking was carried out using iGEMDOCK software. The docked poses were analyzed based on fitness scores. For each protein target, top five compounds showing high fitness scores were selected and their interactions with respective drug targets were analyzed by post screening analysis using Rasmol. Protease was found to be the most potential drug target of HIV-1. Quercetrin from onion and Aloin from aloe plant were found to be the most effective drug candidates for the treatment of HIV infection.

KEYWORDS: HIV, *In-silico* analysis, drug targets, docking and antiviral compounds.

INTRODUCTION
The HIV is a lentivirus, a subgroup of retrovirus that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells[1]. HIV-1 is the most widespread type worldwide which is related to viruses found in chimpanzees and gorillas living in Western Africa[2][3]. As yet, there is no satisfactory or curative treatment for this disease. GP120, GP41, Protease and Reverse transcriptase are the potential drug targets of HIV-1. The GP120 (PDB id: 2me2), molecule of HIV-1 is a glycoprotein part of the outer layer of the virus. It present on the viral membrane spikes consisting of 3 molecules of GP120 linked together and anchored to the membrane by GP41 protein. GP120 is essential for viral infection; it facilitates HIV entry into the host cell[4]. GP41 (PDB id: 5mp6) known as glycoprotein 41 is a subunit of the envelope protein complex of retroviruses, including human immunodeficiency virus (HIV). It is a transmembrane protein that contains several sites within its ectodomain, required for infection of host cells. Its importance in host cell infection, it has received much attention as a potential target for HIV vaccines. In a free virion, the fusion peptides at the amino termini of GP41 are buried within the envelope complex in an inactive non-fusogengic state, stabilized by a non-covalent bond with GP120[5]. HIV-1 protease (PDB id: 1hhp) is a retroviral aspartyl protease (retropepsin) is essential for the life-cycle of HIV, the retrovirus that causes AIDS. HIV protease cleaves newly synthesized polyproteins (namely, Gag and Gag-Pol) at the appropriate places to create the mature protein components of an infectious HIV virion. Without HIV protease, HIV virions remain

uninfectious[6]. A reverse transcriptase (RT) (PDB id: 3klf) is an enzyme used to generate complementary DNA (cDNA) from an RNA template, a process termed reverse transcription. It is associated with retroviruses. Retroviral RT has three sequential biochemical activities, RNA-dependent DNA polymerase activity, ribonuclease H, and DNA-dependent DNA polymerase activity. These activities are used by the retrovirus to convert single-stranded genomic RNA into double-stranded cDNA which then can integrate into the host genome, generating a long-term infection[7]. ART is the combination of several antiretroviral medicines used to slow the rate at which HIV multiplies in the body. A combination of three or more antiretroviral medicines is more effective than using just one medicine to treat HIV[8]. ART is currently the standard treatment for HIV infection. So far, this treatment offers the best chance of preventing HIV from multiplying, which allows your immune system to stay healthy. The goal of antiretroviral therapy is to reduce viral load to a level that can no longer be detected with current blood tests[9]. However there are side effects of the ART. Alternatively, the natural antiviral compounds from medicinal plants can be used for treating HIV infection.

Medicinal plants have been widely used to treat a variety of infectious and non-infectious ailments. Several plants could offer a rich reserve for drug discovery of infectious diseases. A variety of medicinal plants have shown promise to treat a number of viral infections, and some of them possess broad-spectrum antiviral activity[10]. Plants and their products can be utilized as a source of new anti-HIV drugs. *Aloe vera*[11], Camu camu (*Mysciarbia dubia*)[12,13], Sutherlandia (*Sutherlandia frutescens*)[14,15], Tumeric (*Curcuma longa*)[16], Garlic (*Allium sativum*)[17-19], Moringa (*Moringa oleifera*)[20-22], Neem (*Azadirachta indica*) [23-24], Onion (*Allium cepa*)[25-27], were chosen for the study based on review of literature. The comprehensive safety, toxicity and clinical studies are needed for these plants before using them effectively as curative and preventive medications against HIV. The objectives of this study are to select natural antiviral compounds from these medicinal plants based on review of literature and to test the effect of these antiviral compounds targeting the proteins of HIV by *in-silico* methods.

MATERIALS AND METHODS

Target proteins

Three dimensional (3D) structures of target proteins of HIV such as GP 120 (2ME2), GP 41(5MP6), Protease (1HHP) and Reverse transcriptase (3KLF) were retrieved from RCSB protein data bank (RCSB PDB)[28].

Natural antiviral compounds from medicinal plants

Thirty six natural antiviral compounds from various medicinal plants such as Neem (*Azadirachtaindica*), Tumeric (*Curcuma longa*), Moringa (Moringa *oleifera*), Camu camu (*Mysciarbia dubia*), Sutherlandia (*Sutherlandia frutescens*), Garlic (*Allium sativum*), Onion (*Allium cepa*), Aloe vera were chosen for the study based on review of literature.

These compounds were retrieved from pubchem[29] database. They were saved in (.sdf) file format and converted into (.mol2) format using Open Babel [30]. These compounds were screened based on Lipinski Rule of Five.[31]

iGEMDOCKING

iGEMDOCKING is used to analyse the interaction between protein and compound. The protein structure may include a ligand and iGEMDOCK can help to quickly define the binding site. Before docking a ligand, there is need to generate 3D ligand file. iGEMDOCK accepts the MDL MOL, SYBYL MOL2and PDB format for ligand files.

Ligand file was prepared in MOL2 format as input of iGEMDOCK. This software was run for 70 generations to know the best score of the compound.

Based on binding energy, hydrogen bonding, electrostatic, van-Der Waal's interactions, post screening analysis was carried out. Rasmol was used for visualization of protein-ligand interactions[33].

RESULTS AND DISCUSSION

The target proteins were retrieved from PDB and structures were analyzed based on their chains and length. The thirty six natural compounds from eight medicinal plants (*Aloe vera*, Camu camu, Sutherlandia, Turmeric, Garlic, Moringa, Neem and Onion) selected from literature were retrieved from pubchem database. The selected natural compounds from literature were screened based on Lipinski Rule of Five for analyzing the ADMET profile that interprets the adverse properties of Absorption, Distribution, Metabolism, Excretion and Toxicity of the compounds. Among these thirty-six compounds, only seven were found to be negative. The rest of the twenty-nine compounds were found to be positive (Table 1).

Table 1: The selected natural compounds from medicinal plants based on Lipinski Rule of Five.

Sl. No.	Compound Name	Pubchem id	Lipinski Rule of Five					Drug likeliness +/-
			Mol. Mass (Da)	H bond donor	H bond acceptor	Log P	Molar Refractivity	
1	(-)-Catechin	73160	290	5	6	1.546100	72.622993	+
2	4-Hydroxy benzyl isothiocyanate	160611	312	5	6	-0.053101	77.145782	+
3	9,10 Anthraquinone	6780	208	0	2	2.462000	59.748989	+
4	1,4 Anthraquinone	31420	208	0	2	2.774999	61.748989	+
5	Allicin	65036	163	1	1	2.097900	47.712791	+
6	Aloin	12305761	418	7	9	-0.891200	101.179047	+
7	Alpha Atlantone	558173	218	0	1	4.214398	69.292984	+
8	Anthocyanins	145858	208	0	1	2.908190	62.579987	+
9	Asparagine	6267	132	5	4	-3.777800	25.494497	+
10	Barbaloin	12305761	418	7	9	-0.891200	101.179047	+
11	Beta Atlantone	181580	218	0	1	4.214398	69.292984	+
12	Canavanine	439202	176	7	6	-3.848430	37.301895	+
13	Ellagic acid	5281855	302	4	8	1.241200	68.454193	+
14	Emodin	3220	270	3	5	1.887221	69.480392	+
15	Eriodictyol	440735	288	4	6	2.215500	71.859695	+
16	Gaba	119	103	3	2	-2.241601	21.996096	+
17	Gedunin	12004512	482	0	7	4.560699	123.778961	+
18	Kaempferol	5280863	286	4	6	2.305300	72.385681	+
19	Myricetin	5281672	318	6	8	1.716501	75.715271	+
20	Naringenin	932	272	3	5	2.509900	70.194893	+
21	Nimbidinin	101306757	442	3	6	2.822500	115.558357	+
22	Nimbidiol	11334829	274	2	3	3.768199	77.193085	+
23	Nimbin	108058	540	0	9	3.922399	137.078033	+
24	Pinitol	164619	194	5	6	-3.180501	40.830990	+
25	Quercetin	5280343	302	5	7	2.010900	74.050476	+
26	Quercetrin	5280459	448	7	11	0.297000	104.862045	+
27	Rutin	5280805	610	10	16	-1.878802	137.495483	-
28	Salannin	6437066	596	0	9	5.291000	154.583145	-
29	Tumerone	558173	218	0	1	4.214398	69.292984	+
30	Vanillylacetone zingiberone	31211	194	1	3	1.922400	53.660789	+
31	Hinokiflavone	5281627	538	5	10	5.239303	140.037018	-
32	Amentoflavone	5281600	538	6	10	4.819602	140.621826	-
33	Sotetsuflavone	5494868	552	5	10	5.122603	145.509064	-
34	Robustaflavone	5281694	538	6	10	4.819602	140.621826	-
35	Isoginkgetin	5318569	566	4	10	5.425603	150.396301	-
36	Cupressuflavone	5281609	538	6	10	3.886339	138.525803	+

Compounds screened by Lipinski Rule of Five (twenty-nine compounds) were selected for docking. Docking was carried out using iGEMDOCK software against four protein targets of HIV-1 (2ME2, 5MP6, 1HHP and 3KLF) that were retrieved from PDB. The docking results of these compounds against each protein were analyzed. The top five compounds that interact with 2ME2 are Quercetrin (-107.1), Cupressuflavone (-106.17), Barbaloin (-101.26), Aloin (-97.4) and Nimbin (-90.11). The top five compounds that interact with 5MP6 are Barbaloin (-91.8663), Cupressuflavone (-89.54), Aloin (-86.506), Quercetrin (-86.0218) and Nimbin (-77.1951). The top five compounds that interact with 1HHP are Quercitrin (-129.46), Aloin (-126.78), Barbaloin (-124.27), Cupressuflavone (-119.85) and Quercetin (-112.8). The top five compounds that interact with 3KLF are Quercetrin (-80.95), Aloin (-75.1957), Barbaloin (-72.186), Gedunin (-68.99) and Cupressuflavone (-68.76). The interaction of the top five high scoring compounds against each protein was analyzed by Rasmol visualization (Figure 2, Figure 3, Figure 4, Figure 5 and Figure 6) and the important amino acid residues that interact with their target proteins were identified. With all this above analysis, overall top five compounds that have strong affinity against potential drug targets of HIV-1 were identified. These top five compounds (Quercetrin, Barbaloin, Aloin, Cupressuflavone and Nimbin) with their fitness scores are listed in table 2.

Table 2: Top 5 compounds showing high fitness scores against target proteins of HIV-1.

Compound	Pubchem id	GP 41	GP 120	Protease	Reverse trancriptase
Quercetrin	5280459	-107.1	-86.02	**-129.46**	-80.95
Barbaloin	12305761	-101.26	-91.87	**-124.27**	-72.186
Aloin	14989	-97.4	-86.51	**-126.78**	-75.19
Cupressuflavone	5281609	-106.1	-91.4	**-119.85**	-68.76
Nimbin	108058	-90.11	-77.19	**-96.79**	-66.24

Among these top five candidate compounds, Quercetrin from Onion showed strongest affinity against protease with the fitness score -129.46. All the five candidate compounds, (Quercetrin, Barbaloin, Aloin and Cupressuflavone and Nimbin) showed strongest affinity against the same protein, Protease. This analysis indicates that among all the proteins considered for the study, protease (1HHP) was found to be the most potential drug target for HIV-1.

Quercetrin from Onion and Aloin from Aloe vera were found to be most effective drug candidates for the treatment of HIV against Protease (1HHP) which is shown in the bar graph (Figure 1).

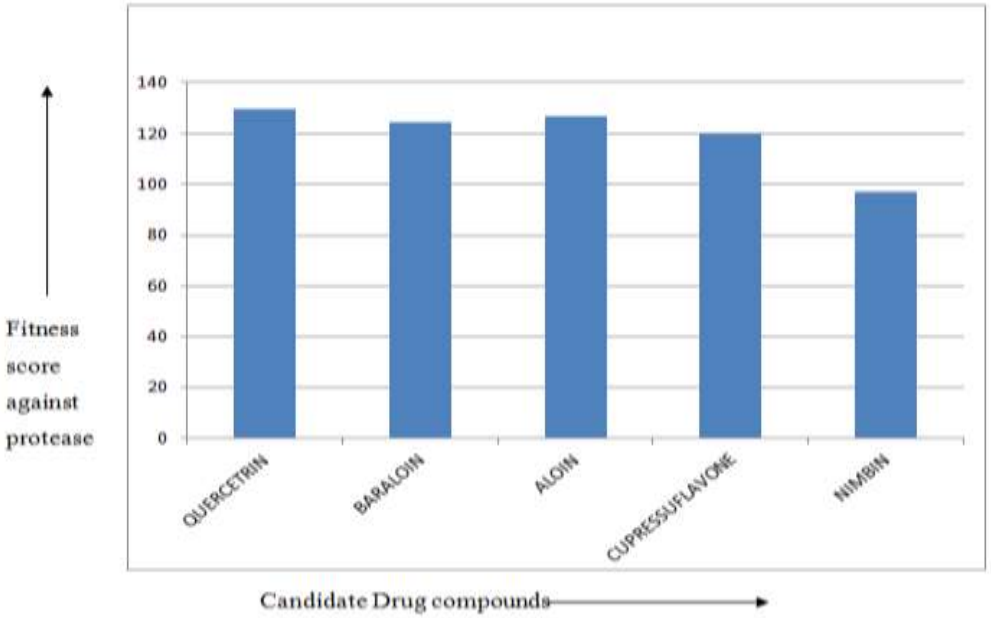

Figure 1: Bar graph showing top five compounds with high fitness scores against Protease of HIV-1.

The structures of post screening analysis for the top five compounds against the proteases of HIV-1 are shown in Fig. 2, Fig. 3, Fig. 4, Fig. 5 and Fig. 6.

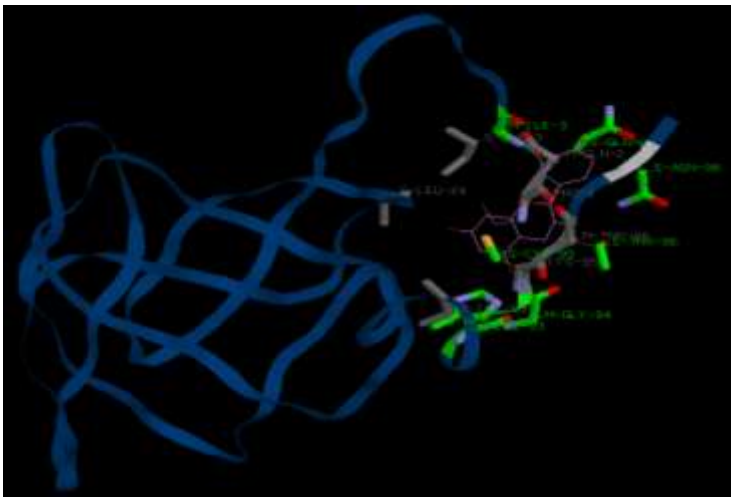

Figure 2: Interaction between Protease and Quercetrin (5280459).

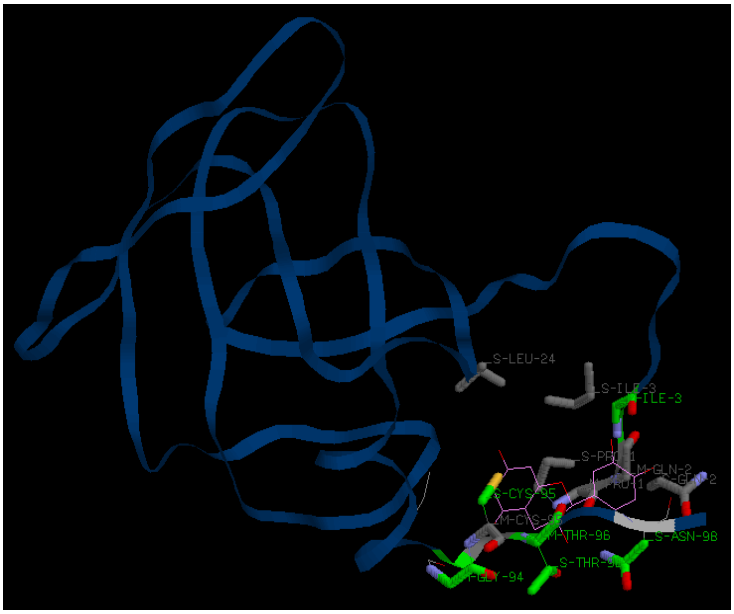

Figure 3: Interaction between Protease and Barbaloin (12305761).

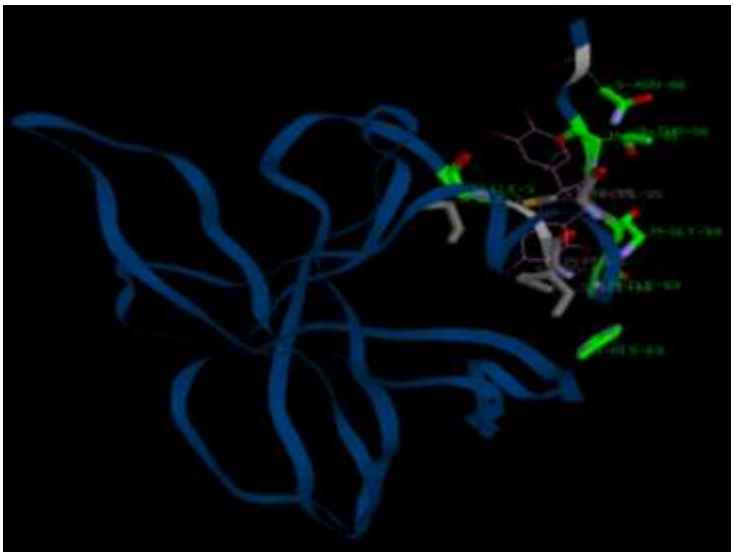

Figure 4: Interaction between Protease and Aloin (14989).

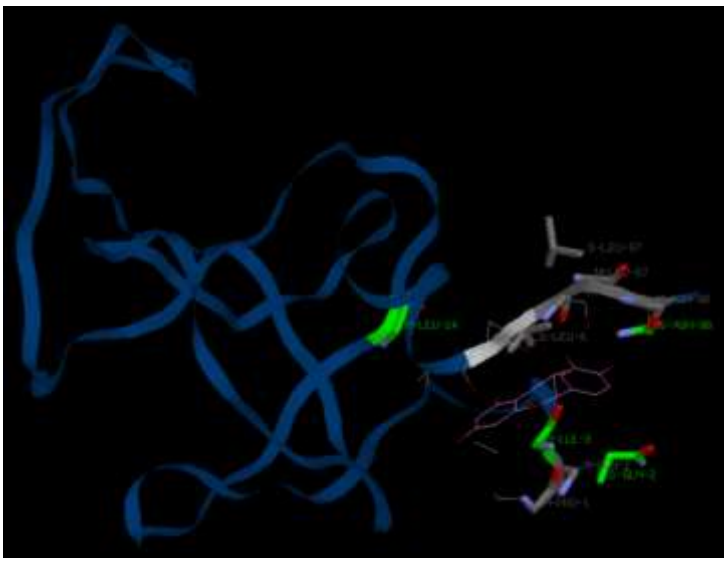

Figure 5: Interaction between Protease and Cupressuflavone (5281609).

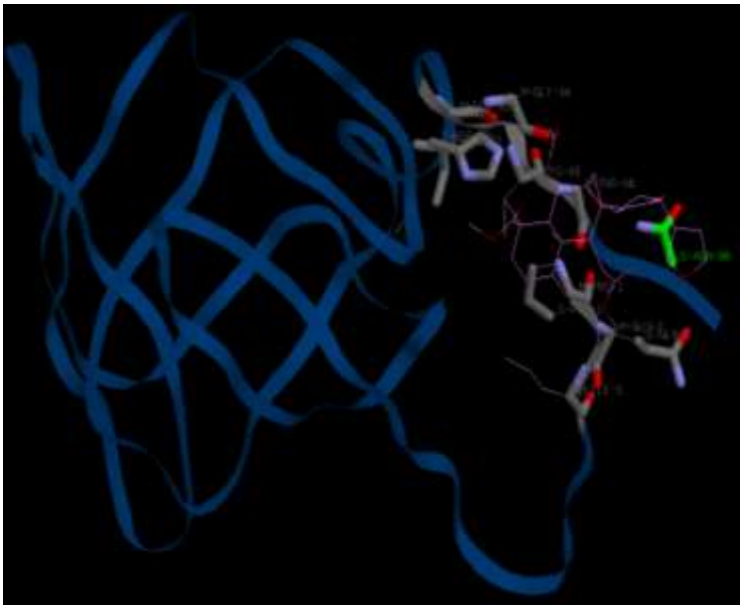

Figure 6: Interaction between Protease and Nimbin (108058).

CONCLUSION

In-silico analysis is one of the safest methods of screening drugs, particularly in viral infections without any side effects. In this study, twenty-nine natural anti viral compounds were analysed by in-silico methods to test their activity against four potential drug targets (proteases) of HIV-1. This analysis indicated that among the proteins considered for this study, Protease (1HHP) was found to be the most potential drug target for HIV-1. Quercetrin from Onion and Aloin from Aloe vera were found to be the most effective drug candidates for the treatment of HIV-1.

REFERENCES

1. Christian Nordqvist, HIV and AIDS: Causes, symptoms, and treatments. Medical News Today, 2017.
2. Joyce K Kikafunda, Hanifa K Namusoke. Nutritional status of HIV/AIDS orphaned children in households headed by the elderly in Rakai district, South western, Uganda. African Journal of Food, Agriculture, Nutrition and Development, 2006; 6(1): 1684-5358.
3. http://en.m.wikipedia.org/wiki/Subtypes_of_HIV#/issues
4. V Yoon, M Fridkis-Hareli, S. Munisamy, J. Lee, D. Anastasiades, L. Stevceva. The GP120 molecule of HIV-1 and its interaction with T cells. Current Medicinal Chemistry, 2010; 17(8): 741-749.
5. Li Huang, Linda Zhang, Chin Ho Chen. Potential drug targets on the HIV-1 envelope glycoproteins, gp120 and gp41. Current Pharmaceutical Design, 2003; 9(18): 1453-62.
6. Marcin Drag and Guy S. Salvesen. Emerging principles in protease-based drug discovery. Nature Review Drug Discovery, 2010; 9(9): 690-791.
7. Kalyan Das and Eddy Arnold. HIV-1 Reverse Transcriptase and Antiviral Drug Resistance (Part 1 of 2). Current opinion in virology, 2013; 3(2): 111-118.
8. https://www.webmd.com/hiv-aids/tc/hiv-highly-active-antiretroviral-therapy-haart-topic-overview
9. Lucia Palmisano and Stefano Vella. A brief history of antiretroviral therapy of HIV infection: success and challenges. Ann Ist Super Sanita, 2011; 47(1): 44-48.
10. Muhammad Mukhtar, Mohammad Arshad, Mahmood Ahmad, Roger J. Pomerantz, Brian Wigdahl and Zahida Parveen. Antiviral potentials of medicinal plants. Virus Research, 2008; 131(2): 111–120.
11. Amar Surjushe, Resham Vasani and DG Saple. Aloe vera: A short review. Indian Journal of Dermatology, 2008; 53(4): 163–166.
12. Paul C. Langley, Joseph V. Pergolizzi, Robert Taylor, Caroline Ridgway, JD. Antioxidant and Associated Capacities of Camu Camu (*Myrciaria dubia*): A Systematic Review. The Journal of Alternative and Complementary Medicine, 2015; 21(1): 8–14.
13. http://www.organichealthandbeauty.com/HIV-AIDS_ep_186-1.html
14. Oluwaseyi M. Aboyade, Gustav Styger, Diana Gibson, Gail Hughes, PH, *Sutherlandia frutescens*: The Meeting of Science and Traditional Knowledge. Journal of Alternative and Complementary Medicine, 2014; 20(2): 71–76.
15. S.M. Harnett, V. Oosthuizen, M. van de Venter. Anti-HIV activities of organic and aqueous extracts of *Sutherlandia frutescens* and *Lobostemon trigonus*. Journal of Ethnopharmacology, 2005; 96 (1–2): 113–119.
16. Soheil Zorofchian Moghadamtousi, Habsah Abdul Kadir, Pouya Hassandarvish, Hassan Tajik, Sazaly Abubakar and Keivan Zandi. A Review on Antibacterial, Antiviral and Antifungal Activity of

Curcumin. BioMed Research International, 2014; 2014.
17. Leyla Bayan, Peir Hossain Koulivand and Ali Gorji. Garlic: a review of potential therapeutic effects. Avicenna Journal of Phytomedicine (AJP), 2014; 4(1): 1–14.
18. Bronwyn G. Hughes and Larry D. Lawson. Antimicrobial effects of *Allium sativum* L. (garlic), *Allium ampeloprasum* L. (elephant garlic) and *Alium cepa* L. (onion), garlic compounds and commercial garlic supplement products. Phytotherapy Research, 1991; 5(4): 154-158.
19. Suman Bukke, Eswari Beeram, M. Venkataswamy and Thyagaraju K. The role of *Allium sativum* (garlic) in various diseases and its health benefits: A comprehensive review. International Journal of Advanced Research (IJAR), 2017; 5(8): 592-602.
20. Garima Mishra, Pradeep Singh, Ramesh Verma, Sunil Kumar, Saurabh Srivastav, K.K. Jha and R.L. Khosa. Traditional Uses, Phytochemistry and Pharmacological Properties of *Moringa olifera* plant- An overview. Scholars Research Library, 2011; 3(2): 141-164.
21. http://glensmoringaoleifera.blogspot.com/2015/04/moringa-treats-hivaids-and-cancer.html
22. Lei Wang, Xiaoyang Chen and Aimin Wu. Mini Review on Antimicrobial Activity and Bioactive Compounds of *Moringa oleifera*. Medicinal chemistry, 2016; 6(9): 578-582.
23. https://www.doctorshealthpress.com/health-articles/the-healing-secrets-of-the-neem-tree/
24. Ugochukwu B. Anyaehie, Medicinal properties of fractionated acetone/water neem(*Azadirachta Indica*) leaf extract from nigeria: a review, Nigerian Journal of Physiological Sciences, 2009; 24(2): 157 -159.
25. Kyu Hang Kyung. Antimicrobial properties of allium species. National Center for Biotechnology Information. U.S. National Library of Medicine, 2011; 23(2): 142-7.
26. Shashank Kumar and Abhay K. Pandey. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal Volume, 2013, Article ID, 16 pages.
27. Robert J Nijveldt, Els van Nood, Danny EC van Hoorn, Petra G Boelens, Klaske van Norren and Paul AM van Leeuwen. Flavonoids: a review of probable mechanisms of action and potential applications. The American Journal of Clinical Nutrition, *2001; 74: 418-25.*
28. https://www.rcsb.org/pdb/
29. https://pubchem.ncbi.nlm.nih.gov/
30. Noel M O'Boyle, Michael Banck, Craig A James, Chris Morley, Tim Vandermeersch, Geoffrey R Hutchison, Open Babel: An open chemical toolbox, *Journal of Cheminformatics*, 2011; **3**(33): 1758-2946.
31. http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp
32. J.-M. Yang and C.-C. Chen, "GEMDOCK: A generic evolutionary method for molecular docking", Proteins: Structure, Function and Bioinformatics, 2004; 55: 288-304.